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Effect of root age on the biomechanics of seminal and nodal roots of barley (*Hordeum vulgare L.*) in contrasting soil environments

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Abstract

Background and aims

The biomechanics of root systems influence plant lodging resistance and soil structural stabilisation. Tissue age has the potential to influence root biomechanical properties through changes in cell wall chemistry, root anatomy and morphology. Within a root system the internal structures of roots are known to vary markedly within different root types. Nodal, seminal and lateral roots of Barley (*Hordeum vulgare*) have differing biomechanical behaviour in tension. This study examines the effects of root age on biomechanical properties of barley root types (*Hordeum vulgare*) under abiotic stress.

Methods

Root age was determined as a function of the distance from root tip with abiotic stresses consisting of waterlogging and restriction to root elongation rate through increased soil bulk density. Linear regression analyses were performed on log-transformed tensile strength and Young's modulus data with best fits determined for single and multiple parameter models to root morphological properties.

Results

Regression co-efficients and Akaike values showed that distance from root tip (taken as a proxy of root age) was the best single variable for prediction of both root tensile strength and Young's modulus. Incorporation of both distance from root tip and root diameter and root type increased the reliability of predictions for root biomechanical properties from 47% to 57% for tensile strength and 35% to 62% for Young's modulus.

Conclusions

The age effect may partly explain some scatter in both Young's modulus and tensile strength to diameter relationship, commonly cited in the literature.

Keywords: Root biomechanics; Root age; Abiotic stress; Soil; Cereal; Modelling

Introduction

Plant roots close to the stem base serve as mechanical anchors in soil that resist lodging from wind stress (Berry et al. 2004). Roots also mechanically reinforce soil (Stokes et al. 2009) and largely drive the formation of soil structure (Hallett et al. 2009). Predicting the extent to which roots influence lodging or soil physical properties, however, is complicated by the complex morphology of root structures, variability of roots between plant species and the large impact of soil properties and time-dependent processes such as root growth and aging (Coppin and Richards 1990; Gray and Ohashi 1983; Loades et al. 2010; Pollen 2007). Loades et al. (2013) provided data on the influence of abiotic stresses on root biomechanics, and went further to demonstrate differences between nodal, seminal and lateral roots of barley. This study supported the statement by Pregitzer (2002) that "a root is not a root", and thus predictions of whole root system mechanical behaviour is extremely challenging.

Most work on the mechanical reinforcement of soils by roots ignores the effects of root type and instead concentrates on root diameter: root tensile strength often decreases with increasing root diameter (Bischetti et al. 2005; Genet et al. 2005; Mickovski et al. 2009; Pollen and Simon 2005). Simple relationships between root diameter and root biomechanical properties are often used in predictive models of soil reinforcement by roots (Loades et al. 2010; Mao et al. 2012; Pollen and Simon 2005; Waldron and Dakessian 1981). Questions have been raised on how accurate the use of diameter is when predicting maximum tensile stress (force per unit area) and Young's modulus (a measure of root elastic properties) with poor fits within some species (Beek et al. 2005). Hales et al. (2009) suggest that relating maximum load (the peak force required for a root to break) directly to root

58 cross-sectional area is one way to eliminate the effects of auto correlation resulting from
59 the use of diameter in calculation of root tensile strength (peak force divide by root cross-
60 sectional area). Very few other studies suggest alternative dependant variables to diameter
61 for predicting biomechanical properties.

62 It is important to consider the influence of the root environment on root anatomy and
63 biomechanical properties. In waterlogged conditions roots may be thicker, develop radial
64 barriers to oxygen loss in outer cell layers of the root, and develop aerenchyma (air channels
65 within the root cortex that improve oxygen transport to the root tip; Garthwaite et al.
66 (2003)). In strong soils, mechanical impedance often causes an increase in root diameter
67 (Bengough et al. 2006). Based on the negative relationship between root strength and root
68 diameter, it therefore follows that biomechanical properties could be compromised in
69 waterlogged or in compacted soils. Such abiotic stresses could lead to a weakened root
70 system potentially resulting in crop lodging and soil de-stabilisation, and compromising yield
71 and long term soil fertility.

72 Studies that sample roots from discrete soil volumes in the field to assess biomechanical
73 properties will undoubtedly collect roots of varying age due to the natural growth and
74 turnover within root systems and depth of sampling. Root turnover rates are dependent on
75 a number of factors including genotype, local soil conditions, location in the soil profile and
76 the type of root system (Gill and Jackson 2000).

77 One of the practical experimental difficulties in studying root age effects is measuring the
78 age of a single root within a population. Roots grow acropetally (Galamay et al. 1992) and, in
79 homogeneous soil environments, cereal root axes may grow up until flowering at a
80 relatively constant rate dependent on the soil conditions around the root tip. Seminal roots

of barley and wheat in nutrient solution and in sand culture have been observed to elongate at an approximately constant rate during their first month of growth (Rose, 1983). There is much less data on whether increase in root length is linear for roots subjected to physical stresses, though linear increase in maximum root depth versus time was recorded for winter wheat grown in the field on both calcareous clay and sandy loam soils (Ellis and Barnes, 1983). Root elongation rate also remained relatively constant with respect to thermal time at the root tip of seminal and nodal axes of maize grown in the field (Pellerin and Pages, 1994). Hence, distance from the root tip may be a reasonable indicator of root age, at least in respect of evaluating its potential importance for influencing root strength. During root elongation the primary cell walls of expanding cells yield from pressure exerted within the cell, with cell wall strength and stiffness increasing further once growth is complete (Gibson 2012).

It is difficult to age root tissue samples from *in situ* specimens, especially when excavation is limited by soil adherence to roots. As roots age, lignin and other structural compounds are deposited and formed in the root cell walls (Kotula et al. 2009). Lignin is deposited within the cell walls and is associated with cell wall thickening (Campbell and Sederoff 1996). Within the roots of rice (*Oryza sativa* L.) lignin and aromatic suberin increase in the outer cell layers of the root with increasing distance from the root apex (Kotula et al. 2009). Distance from root tip can therefore be used as a relative measure of root tissue age; sections nearest the tip are the youngest with those furthest away the oldest. It is not possible to ascribe a precise age to any particular section of root tissue, as plant root extension rate is influenced by soil conditions (Bengough et al., 2006; Watt et al., 2006; Watt et al., 2003). There is an initial acceleration of root growth in the few hours or days

following germination or the initiation of a root axis (e.g. in maize; Blacklow, 1972), and there may be a tendency for successive nodal axes to grow at slightly increased rates of extension (e.g. in millet; Gregory, 1986). However, the distance from root tip provides an easily attainable *relative* indicator of root age that should evaluate the likely importance of root age as a factor in determining root biomechanical properties.

Experiments conducted within this paper aim to validate the effects of distance from root tip, root type and the soil environment on root tensile strength and Young's modulus. Furthermore, potential influences of mechanical impedance and transient waterlogging are studied. Using linear regression analysis we studied the effects of root age (distance from root tip), root diameter, root type (nodal or seminal) and soil treatment (normal, waterlogged or mechanically impeded) on root tensile strength and Young's Modulus. The research has direct relevance to the development of models for predicting the reinforcement of soil by plant roots and to the understanding of root-soil mechanical interactions that drive lodging resistance.

Methods

Plant growth conditions

Plants were grown in soil packed into 1 m length x 0.05 m diameter plastic tubing that was lined with 0.2 mm thick plastic sheeting to ease the removal of soil from the tubes at the end of the experiment. The soil was an arable sandy loam (Eutric Cambisol) consisting of 71% sand, 19% silt and 10% clay, with a pH of 6.2 (White et al., 2000) and sieved to 4 mm. A layer of pea gravel, 20 mm thick, was packed in the base of tubes with a further 980 mm of

soil above. Soil was packed to a dry bulk density of 1.2 g cm^{-3} for control and waterlogged treatments and an increased density of 1.4 g cm^{-3} for roots subjected to degree of mechanical impedance. Soil was wetted to 0.20 g g^{-1} water content and allowed to equilibrate for 24 h prior to packing. Packing was performed using a proctor hammer with a mass of 2.78 kg dropped from 20mm, to achieve a soil density of 1.2 g cm^{-3}, and 80 mm to achieve a density of 1.4 g cm^{-3}. The energy required for packing control and waterlogged soil was 7 kJ m^{-3}, and 113 kJ m^{-3} for the mechanical impedance treatment, with soil packed in 25 and 50 layers respectively. Energy for packing was calculated based on the number of blows for each layer from a known mass dropped from a fixed height (Loades et al., 2013).

Barley (*Hordeum vulgare* cv. Bowman) grains were sterilised in 2% saturated $Ca(ClO_2)$ for 15 mins, washed in sterilised distilled water and left for 3 d at 12^{o}C on filter paper in the dark to germinate. Single germinated seedlings were planted in the centre of each tube of soil at a depth of 10 mm in a pre-bored hole. Soil was then replaced over seedlings following planting. Each treatment was replicated four times with plants grown in a controlled environment at 18^{o}C receiving 16 h of light (300 µmol m^{-2}) and 8 h of darkness over a 24 hr period. Four soil tubes were then grouped and placed together in larger outer (160 mm diameter) tubes. For water-logged treatments the bases of the soil tubes were left open, whereas for the control and mechanically impeded treatments the soil tubes were sealed with screw caps to prevent water ingress. All of the outer tubes were filled with water to ensure temperature buffering effects were the same between treatments. Waterlogging was applied 7 d after emergence, followed by drainage, and then a further 7 d of growth (for full details see Loades et al. (2013)).

149 *Harvesting and biomechanical testing of roots*

150 Planting of germinated seeds was staggered to ensure that roots were mechanically tested
151 within 3 days of harvesting. Due to the time required for sample preparation it was not
152 practical to plant and sample all plants at once. Plants were harvested 21 days after sowing
153 with soil columns removed from pipes by pulling on the plastic sleeves. Once removed, tap
154 water was used to gently wash away soil from the roots over a 2 mm sieve. Extra care was
155 taken to reduce the risk of root mechanical damage. Only intact roots from the stem base
156 with a clearly defined growing root tip were used for mechanical testing. Following washing,
157 roots were sectioned into lengths at least 60mm long with distance from root tip recorded.
158 Following washing, roots were stored at 5^{o}C on moist blotting paper. Immediately prior to
159 testing, root diameter at the mid-point of root sections was measured using an eye-piece
160 graticule with 10 X objective on a Leica MZFLIII stereo microscope (Leica, Milton Keynes,
161 United Kingdom). The root biomechanical properties tensile strength and Young's modulus
162 were calculated based on cross-sectional area derived from root diameter measurement
163 with a graticule and microscope prior to testing.

164 An Instron 5544 universal test frame (Instron, Norwood, MA, USA) was used to mechanically
165 test roots under tensile loading using an axial extension rate of 1 mm min^{-1}. Samples were
166 secured using screw side action grips positioned 40mm apart, allowing a minimum 10 mm of
167 root section to be fixed in each clamp. Extension was recorded through cross head
168 displacement with load measured using a 50N load cell with ±2 mN accuracy. Tensile
169 strength (maximum tensile stress at failure) was calculated as peak force divided by root
170 cross-sectional area. Young's modulus was derived from the initial gradient of the stress-
171 strain plot during tensile testing within the elastic region. Variables investigated in this study

were: root diameter; distance from root tip; soil condition (normal, mechanically impeded or waterlogged); and root type (nodal or seminal).

Statistics

All data were analysed using GenStat (15th edition) statistical software. Root tensile strength and Young's modulus data were Log_{10} transformed in order to obtain a normal distribution, allowing data analysis using linear models. An all sub-set regression analysis was performed to investigate the contributions of each predictor variable (treatment, root type, distance from root tip or diameter) to root tensile strength and to Young's modulus. The all sub-set regression analysis produced linear fits for each predictor variable to tensile strength and Young's modulus data, displaying the best-fitting models containing one predictor variable, two predictor variables etc. This allowed comparison between the relative importance of each predictor variable for the optimal prediction of tensile strength or Young's modulus. Outputs from the regression analyses produced both r^2 values and also Akaike Information Criterion (AIC) values enabling assessment of each predictor variable and its contribution to strength and modulus. AIC values represent a numerical index that can be used to compare several competing models, with the smallest AIC value representing the model with fewest parameters that best describes the data (Symonds and Moussalli 2011). The AIC value is based on information theory, and rates models more highly if they have a good fit to the truth (minimising the Kullback-Leibler distance) whilst penalising them if they contain many parameters (Burnham and Anderson, 2002). AIC values were calculated using:

$$AIC = deviance/f + 2*r \,, \qquad (1)$$

194 Where deviance is similar to log-likelihood, *f* = the dispersion parameter (relating to how
195 widely dispersed the data is) and *r* = the numbers of parameters fitted in the current model.
196 Each variable is fitted in turn to the property being examined (e.g. tensile strength) and the
197 best single variable for predicting this chosen (independent of the original predictor variable
198 order). Using this most influential variable as a base Genstat investigates which of the
199 remaining variables improves the fit, this improvement is quantified by improvements in
200 both the r^2 value and also a decrease in the Akaike value. Reductions in the AIC value, with
201 each new variable added to the model, highlights improvements in model fit: increases in
202 AIC indicate a worsening of model fit in relation to the number of parameters used.
203 Unbalanced analysis of variance tests were performed on root diameter data for root
204 sections of different ages (distances from root tip).

205

206 **Results**

207 Within all of the treatments there were significant differences in root diameter between
208 nodal and seminal roots (Table 1). Distance from root tip influenced the diameter of seminal
209 roots grown in both the control and soil of an increased density treatments (Table 1;
210 $P<0.001$). When all roots within each treatment were grouped together, distance from root
211 tip was also found to influence root diameter within all treatments (Table 1). Seminal roots
212 grown in the control soil and soil of an increased density were of thickest diameter near the
213 root apex (i.e. youngest section) than in the older tissue close to the stem base (Figure 1).
214 Waterlogging resulted in seminal root die back with increased nodal root growth so seminal
215 root properties from waterlogged soils were not included in seminal root analysis.

Root tensile strength of all root types decreased with increasing root diameter with root strength significantly affected by root diameter in both control ($P<0.001$) and 1.4 g cm^{-3} soils ($P<0.001$; Figure 2.). Within waterlogged samples root strength did not show any significant relationship with diameter ($P=0.054$). Similar to root strength, Young's modulus decreased with increasing root diameter with modulus significantly affected by root diameter in all treatments ($P<0.001$; Figure 3.). Root tensile strength and Young's modulus in the control and 1.4 g cm^{-3} soils increased with increasing distance from root tip ($P<0.001$) but not in the waterlogged treatment ($P=0.632$). Dumlao et al. (2013) have also observed increases in root tensile strength with increasing distance from root tip.

Root tensile strength was most closely related to distance from root tip (the best fit for a single variable model) ($r^2 = 0.468$) with the inclusion of diameter improving fit for a two variable linear model ($r^2=0.538$; AIC value decreased from 285.5 to 250.5). Within the three and four variable models, AIC values further decreased, signifying an improved fit with the additions of root type and treatment decreasing from 236.5 to 222.0 respectively (Table 2).

Similar to results for the root tensile strength model, predictions of Young's modulus in a single variable model found distance from root tip was the best fit ($r^2 = 0.345$) with further improvements with the addition of diameter in a two variable model ($r^2=0.495$; AIC value decreased from 366.5 to 285.8). Adding root type decreased the AIC value, and increased r^2 further, in a three variable model decreasing from 285.8 to 218.0. The inclusion of treatment in a four variable model increased the AIC value indicating that the fit was not as robust as the three variable model (Table 2).

Discussion

Distance from root tip (root age proxy) had a major effect on root diameter and biomechanical properties, and this effect depended on both the soil environment and the root type. Nodal roots did not change in diameter as they aged, although it was only in the waterlogged treatment that nodal roots longer than 40 cm long were present. Older regions seminal roots in the control and 1.4 g cm^{-3} density soil were thinner by up to 30 to 40%. This thinning of root axes with age could be associated with the decrease in turgor of cells in the root cortex (Bingham 2007). Bingham found that a thinning of seminal root diameter from 0.9 mm, 20 cm behind the root apex, to 0.25 mm 80 cm behind the root apex, was associated with the percentage of turgid cells decreasing from >90% to 0% in the cortex of seminal roots of winter wheat.

Root tensile strength is widely assumed to be diameter dependant, with predictions typically derived from fitting negative power-law curves to mixed populations of roots sampled. The accuracy of these fits has shown to be reasonable within woody species by some researchers (Bischetti et al. 2005; Genet et al. 2005; Mickovski et al. 2009) but, in other papers, the fits appear to be poor (Beek et al. 2005; Genet et al. 2008; Mattia et al. 2005). In fibrous root systems the fit between root strength and diameter, or Young's Modulus with diameter, has been shown to be variable. Plants grown in controlled environments produce much better correlations between mechanical properties and diameter, when compared with field grown plants, with changes in measured shear strength dependent on the time after sowing (Loades et al. 2010). For Young's modulus, our results also showed distance from root tip (Figure 3) to be the best single variable predictor.

Regression models highlighted the effect of root age, as a function of distance from the root apex, on root biomechanics (Table 2). Both single variable models, for tensile strength and Young's modulus were most closely related to distance from root tip. This finding is consistent with previous work by Easson et al. (1995), where root strength decreased with increasing distance from the stem base, albeit limited to roots at most 12 cm from the stem base. A thigmomorphogenic response may be expected close to the stem base due to mechanical perturbation of the shoot by wind and rain (Jaffe 1973). Due to the relatively long length of roots examined in our study, and the absence of mechanical perturbation from the controlled growth conditions, thigmomorphogenesis would have little impact on the results. However, some thigmomorphogenic stimulation may also occur as root tips push past and contact soil particles –however roots grown in hydroponics have previously been shown to elicit similar biomechanical properties to those grown in compacted soil (Loades et al, 2013).

The increase in the Young's modulus and root strength with increasing distance from the root tip may be linked to cellulose content and also the deposition of lignin during tissue development. Hathaway and Penny (1975) reported that Young's modulus was positively correlated with cellulose and decreased with increasing lignin/cellulose ratio in some *Populus* and *Salix* clones. In Martime pine and Sweet chestnut, root tensile strength increased with increasing relative cellulose, which was more abundant in thinner roots (Genet et al. 2005). More recently, examination of other woody species (Douglas fir and European beech) have shown a positive increase in cellulose with increasing diameter and negative relationships between lignin and root diameter (Thomas et al. 2014). Within maize

roots, lignin content increases along the root within both endodermal and hypodermal cell walls (Zeier et al. 1999), but no information is available for barley.

In rice roots, segmented into 10 mm sections from the root apex, lignin and suberin content increased with age (Kotula et al. 2009). Lignin and suberin contents were also greater for growth in aerated versus deoxygenated hydroponics (Kotula et al. 2009). These observations highlight the impact of abiotic stress on root tissue chemistry and may explain differences in biomechanical properties reported here and previously (Loades et al. 2013).

Modelling parameters currently used, are they adequate?

Previous research has shown that root diameter significantly affects strength (Genet et al. 2005; Loades et al. 2010; Pollen 2007). Although including diameter within a two variable model increased the goodness of the fit for both tensile strength and Young's modulus, distance from the root tip provided an even better fit. These findings suggest that models of root reinforcement (e.g. Pollen and Simon, 2005) or root anchorage (e.g. Berry et al. (2006)) could be improved by incorporating root age effects along the length of roots. Moreover, age related information on root biomechanics could be combined with root growth models to simulate how reinforcement by whole root systems may vary over time.

Conclusions

Root strength was more closely related to distance from the root tip (root age) than root diameter for both nodal and seminal roots of barley, as analysed using a single variable

303 linear model. The finding was robust for roots grown in control, compacted, or waterlogged
304 treatments. These results could explain some of the uncertainty observed in modelling root
305 reinforcement or anchorage from diameter versus strength relationships alone, suggesting
306 that root age effects should be incorporated into models.

307 The influences of age and environment are probably associated with lignin and cellulose
308 deposition within roots. This also suggests that there may be substantial variation in root
309 strength between genotypes due to variation in both structure and composition. Further
310 work should also examine declines in root strength as plants senesce to enable longer term
311 predictions of soil stability throughout and between growing seasons.

312

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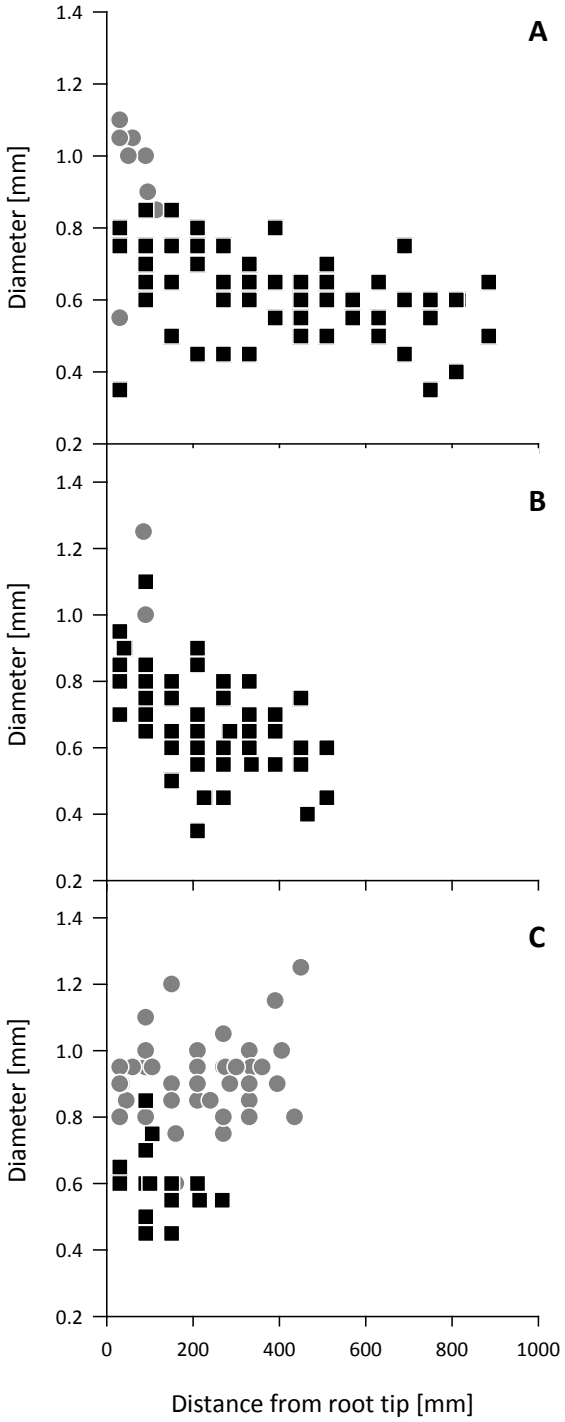
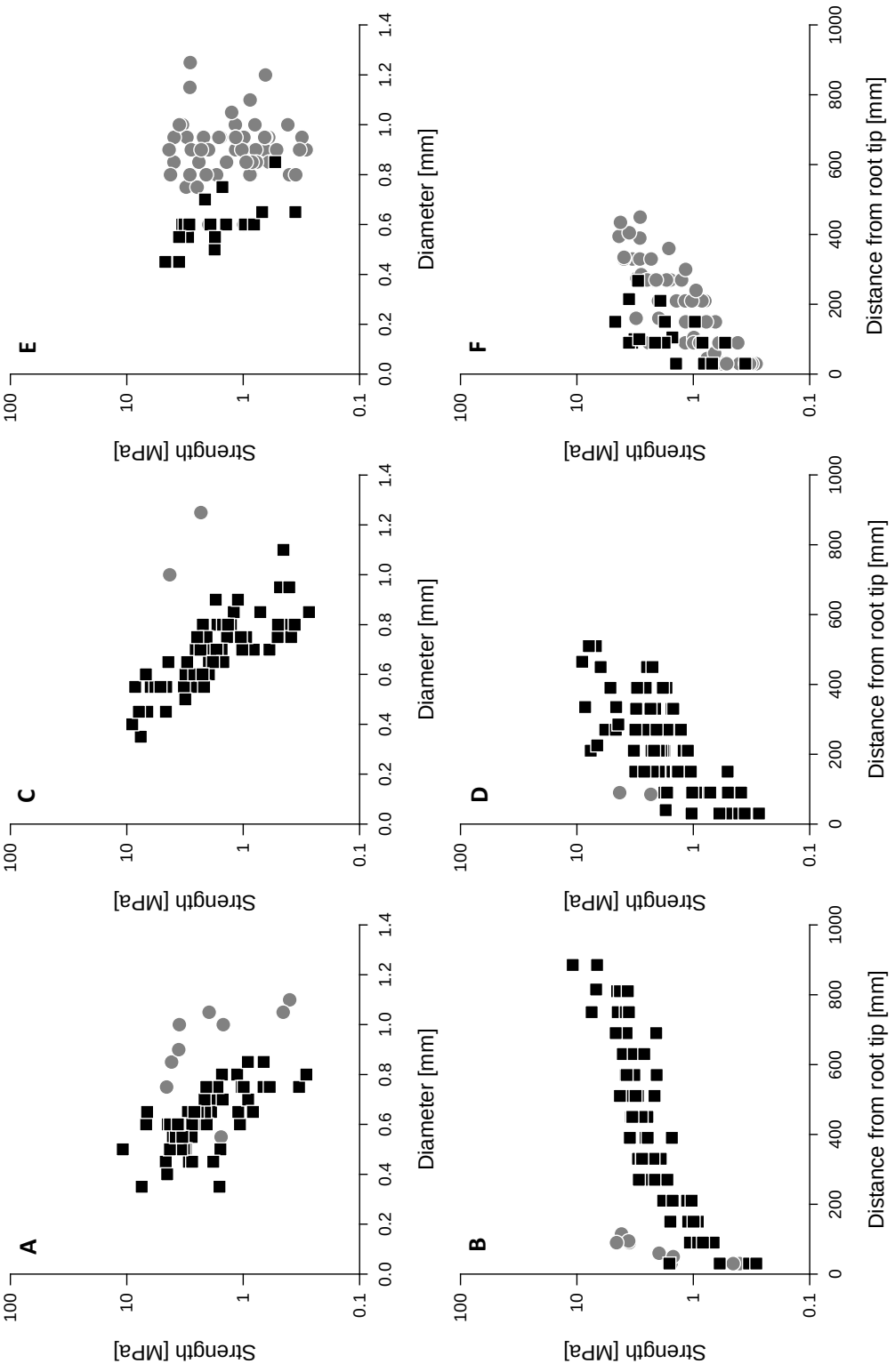
317 **Figures**



318

319 **Figure 1:** Root diameter as a function of distance from root tip for control (**A**), mechanically
320 impeded (**B**) and waterlogged treatments (**C**); ■ = seminal roots, ● = nodal roots

321



322

323 **Figure 2:** Root tensile strength as a function of diameter and distance from root tip grown in
324 control soil (**A** and **B**), under mechanical impedance (**C** and **D**), and when subjected to
325 waterlogging (**E** and **F**); ■ = seminal roots, ● = nodal roots

326

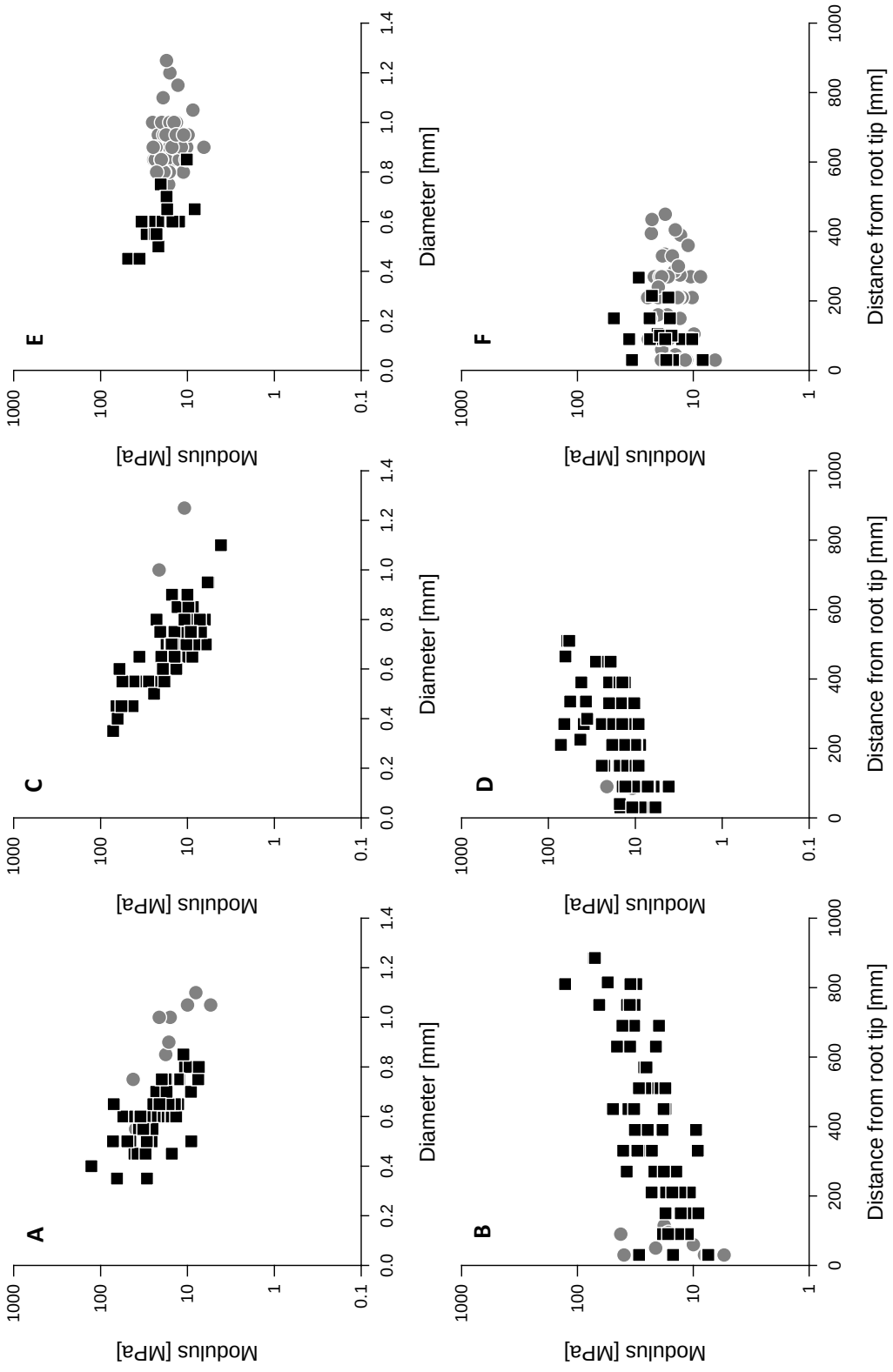


Figure 3: Young's modulus as a function of diameter and distance from root tip grown in control soil (**A** and **B**), under mechanical impedance (**C** and **D**), and when subjected to waterlogging (**E** and **F**); ■ = seminal roots, ● = nodal roots

Tables

Table 1: Mean root diameter for each root type grown in control, waterlogged and mechanically impeded conditions. Within all treatments there were significant differences in root diameter dependant on root type (P<0.001).

	Control			***Mechanically impeded***			***Waterlogged***		
Root type	**Mean diameter [mm]**	**Standard error**	**Significance of distance from tip on root diameter**	**Mean diameter [mm]**	**Standard error**	**Significance of distance from tip on root diameter**	**Mean diameter [mm]**	**Standard error**	**Significance of distance from tip on root diameter**
Nodal	0.917	0.06	P=0.736	n/a	n/a	n/a	0.906	0.02	P=0.091
Seminal	0.617	0.01	*P<0.001*	0.686	0.02	*P<0.001*	0.603	0.02	P=0.242
All roots	0.654	0.02	*P<0.001*	0.699	0.02	*P<0.001*	0.830	0.02	*P<0.01*

336

337 **Table 2:** Results of all subset regression analyses, Akaike and r^2 values reported to describe
338 fits. Linear regression applied following log transformations of tensile strength and Young's
339 modulus data (all models have 2 degrees of freedom).

340

		Single term Distance from tip	**2 term + diameter**	**3 term + root type**	**4 term + treatment**
Tensile Strength	r^2	0.468	0.538	0.568	0.603
	Akaike	285.5	250.5	236.5	222.0
Young's Modulus	r^2	0.345	0.495	0.621	0.621
	Akaike	366.5	285.8	218.0	222.0

341

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